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Editorial note:

In this issue of Topical Update, Dr. Janette KWOK introduced to us on the immunogenetics. This is an area of great potential in understanding the immune response which is encoded in our genetic makeup and therapeutic choices. We welcome any feedback or suggestions. Please direct them to Dr. WK Luk (e-mail: lukwk@ha.org.hk) of Education Committee, the Hong Kong College of Pathologists. Opinions expressed are those of the authors or named individuals, and are not necessarily those of the Hong Kong College of Pathologists.

Immunogenetics: MHC and non-MHC

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Introduction

Immunogenetics is the study of the immune response in relation to genetic makeup. The immune system protects the vertebrates from all potential harmful infectious agents such as bacteria, virus, fungi and parasites. The growing understanding of the immune system has influenced diversified biomedical disciplines, and is playing a significant role in the study and treatment of many diseases such as cancer and autoimmune conditions.

The launch of immunogenetics could be traced back to the demonstration of Mendelian inheritance of the human ABO blood grouping in 1910. Major developments leading to the

emergence of immunogenetics were accounted by the rediscovery of allograft reactions during the Second World War and the formulation of the immunological theory of allograft reaction and the clonal selection hypothesis by Burnett in 1959.

The field of immunogenetics has exploded during the last 25 years, thus expanding the range of concepts with the potential to improve the field of medicine with regard to transplantation, immunotherapy and the study of immune polymorphisms. Immunogenetics has poised on the brink of a new era, driven by the development of new technologies and shaped by fundamental discoveries about the mechanisms that regulate interactions between the adaptive and innate immune systems. Technologies are developed to

revolutionize genetic analysis and providing new strategies for elucidating the genetic mechanisms that influence immune responsiveness and autoimmunity.

The most relevant influence on the development of immunogenetics is the studies of the highly polymorphic gene family well known as the major histocompatibility complex (MHC). These genes were first studied as antigens of the white blood cells and hence named human leukocyte antigens (HLA). They have influenced both the donor choices in organ transplantation and the susceptibility of an organism to chronic diseases. The MHC is also linked with many important autoimmune diseases such as rheumatoid arthritis and diabetes.

The explosion in immunogenetics studies of these molecules was ultimately resulted from the discovery in 1972 that these MHC molecules are intimately associated with the specific immune response to viruses. This has led to the construction of very detailed genetic and physical maps of this complex and its complete sequence in an early stage of the great human genome sequence project.

The target for this update is to bring out a brief overview of some of these new areas that are of clinical importance to the field of HLA and immunogenetics, and hopefully provide some thought into the future of modern methods and their potential importance in understanding the immune response.

Non-classical MHC Class I

The impact of non-classical HLA molecules in the immune response has been investigated only in these recent years. Humans encode three “non-classical” MHC Class I genes, HLA-E, F, and G. These relatively new class I genes and molecules were first described by Koller and colleagues in 1989. In general, these non-classical HLA molecules are considered immune “tolerization” molecules, which not only interact with natural killer (NK) cells but also with T lymphocytes and other cells. These non-classical class I molecules are similar in sequence and structure to MHC

class I molecules but do not show the striking polymorphism seen for their classical relatives and may be encoded outside the MHC. These molecules are characterized by unique patterns of transcription, protein structure and immune function.

The study of HLA-E in transplantation has yielded information regarding the pattern of HLA-E expression suggesting its important role in immune recognition. HLA-E molecules may present some peptides, such as MHC signal sequences, to T lymphocytes and this recognition is mediated by the interaction of HLA-E with the CD94/NKG2 receptor and can result in either ‘inhibition’ or ‘activation’ of the NK cell.

Several recent studies have shown how HLA-E and HLA-G act as potential powerful modulators of the innate immune response with regard to susceptibility to infectious processes. HLA-F was identified at the same time as HLA-G and HLA-E but there is little information to date about its expression and function. HLA-F is only slightly detectable on the cell surface of trophoblast, some B and mononuclear cell lines and occurs predominantly as an intracellular, empty and unstable class I protein. On peptide binding, HLA-F is expressed and can interact with immunoglobulin-like receptors ILT2 and ILT4, leading to an altered activation of immune effector cells. The unusual characteristics of the predicted peptide-binding groove of HLA-F, together with its predominantly intracellular localization, raises the possibility that HLA-F might be capable of binding only a restricted set of peptides.

Tumor cells have been found to express the non-classical HLA molecules. Evidence suggests a possible role of these molecules in immune recognition. Abnormalities in non-classical MHC expression have been found in human tumors and might lend insight into clinical outcome. Implications that a delicate balance needs to exist between stimulating and suppressing signals among cells expressing non-classical MHC suggest that studying this immune response could provide information regarding possible tumor progression.

MHC Class I-related chain genes

In 1994, two new polymorphic families of MHC class I-related genes, termed MHC class I-related chain A (MICA) and B (MICB) which were contained within the MHC region, were described and already sparked a new area of interest in the HLA community in relation to transplantation. These genes are located near the HLA-B locus on chromosome 6 and encode cell surface glycoproteins that do not associate with β -2 microglobulin. These molecules function as restriction elements for intestinal γ/δ T cells and they behave as cell stress molecules. MICA is expressed in endothelial cells, keratinocytes and monocytes, but not in lymphocytes, thus making them potentially important in solid organ transplant. It is likely that the polymorphic MICA molecule may be a target for specific antibodies and T cells in solid organ grafts or in graft vs. host disease (GVHD).

MIC molecules interact with both T cell and NK cell receptors. MIC antigens have been implicated in transplant rejection because anti-MIC antibodies are often found in transplant recipients, reminiscent of the classical anti-HLA antibodies. These antibodies may facilitate some complement-mediated cytotoxicity against endothelial cells from the graft well documented in a four year follow up study of a prospective trial in kidney transplants have provided strong evidence that HLA and MICA antibodies are associated with graft failure. The anti-MICA antibodies were found to induce a prothrombotic state, characterized by a loss of surface heparan sulphate and thrombomodulin from cultivated endothelial cells.

Minor Histocompatibility Antigens

A different set of polymorphic non-MHC proteins have been identified that are important in provoking transplant rejection, they were defined by Snell and colleagues as minor histocompatibility antigens (mHAg), as the rejection reactions they induced in mice were slower. Peptides from these proteins are presented to T cells in an MHC class I or class II restricted manner. The role of mHAg as the facilitators of

GVHD as well as the targets for immunotherapy of cancer was well studied by Prof. Els Goulmy. The number of possible mHAg in transplants performed between genetically unrelated, MHC-matched individuals is very large. However, the reactions seem to be restricted to a few epitopes, thus dubbed immunodominant. The molecular basis for this phenomenon is incompletely understood, although it has recently been shown that both the duration of individual mHAg presentation and the avidity of T-cell antigen recognition influence the magnitude of the cytotoxic response that ensues. Though mHAg are named minor, and the frequency of responders to these antigens is very low, after transplantation, a single immunodominant mHAg can induce GVHD. Apart from gene polymorphisms, homozygous gene deletions can also serve as mHAg as it has recently been described for an autosomal gene in the UDP-glycosyltransferase 2 family. mHAg were originally identified as a plausible explanation for the cause of graft rejection or GVHD in HLA-matched allogeneic haematopoietic stem cell transplants (HSCT).

Molecular identification has revealed that most mHAg are short peptide fragments encoded by genes, which are polymorphic due to single nucleotide polymorphisms (SNPs). Disparity at the genotypic level between donor and recipient gives rise to mHAg as non-self antigen differences for both the donor and recipient. Thus, information gathered from both solid organ and HSCT could provide information regarding the importance of this system's immune function. Identification of mHAg currently employs PCR-based methods.

When minor antigens are tissue restricted, they can be considered an adjunct for graft vs. tumor responses in HSCT. This strategy is currently under investigation in phase I/II clinical trials in which post-transplant recipients are boosted with donor lymphocyte infusions using tumor-specific minor peptides. There are trials for hematologic malignancies using mHAg HA-1 and HA-2, and for breast and renal cell carcinoma using HA-1. The integration of clinical practice of the tolerizing potential of minor antigens in solid organ transplantation is well demonstrated by the

HA-1-specific alloimmune responses may lead to allograft tolerance in renal transplantation. HA-1-specific T regulator cells can be identified in long-term transplant recipients and correlate with HA-1-specific cytotoxic T-lymphocyte responses and microchimerism. As such, they provide a potential strategy for optimizing immunosuppressive therapy by monitoring HA-1 responses.

Projects in the field of mHAg have been continued in 13th, 14th and the soon-to-be 15th International Histocompatibility and Immunogenetics Workshops (IHIWS). Data is being gathered to formalize information pertaining to this system.

Killer Cell Immunoglobulin-like Receptors

The killer cell immunoglobulin-like receptor (KIR) genomic region displays extensive diversity through variation in gene content and allelic polymorphism within individual KIR genes. Family segregation analysis, genomic sequencing and gene order determination proven that genomic diversity have already given rise to more than 20 different KIR haplotypes and 50 KIR genotypes. The importance of this recognition stems from the fact that in the clinical setting of mismatched HSCT, donor vs. recipient NK cell alloreactivity has been associated with better outcome. This alloreactivity derives from a mismatch between inhibitory receptors for self-MHC class I molecules on donor NK clones and the MHC class I ligands on recipient cells. NK cell function is regulated by clonally distributed inhibitory receptors that are specific for self-MHC class I molecules. Lack of engagement of these receptors results in target cell lysis (missing self-recognition), which has the potential to eliminate the remaining malignant recipient-originated cells. The National Marrow Donor Program (NMDP) high-resolution KIR typing pilot project was initiated to evaluate the efficacy of performing allele level KIR typing.

The role of NK cell alloreactivity in solid organ transplantation is less known. Results in animal models show that NK cells are neither necessary nor sufficient for acute immune rejection - which does not exclude an NK cell contribution to the

rejection process. In addition, work continues in determining the importance of the KIR system in many aspects important to medicine, such as impact on many disease states and the risk of viral function. Recent work on the KIR system with regard to viral function could provide information on how to combat certain viruses. These areas of interest have pushed the field forward to develop different commercial methods for KIR typing.

Cytokine Polymorphism

Cytokine polymorphism and signaling is also becoming a major focus for understanding and interpreting the immune response. Cytokines are secreted molecules which act on their surrounding environment to help provide cell-to-cell signaling, affecting not only in HSCT but also other immune modulated environments.

The “cytokine storm” which occurs when subjecting cells to different conditioning regimens in HSCT or solid organ transplant must have diverse effects on this microenvironment. The monitoring of this environment could be essential in adjusting the post-transplant immunotherapy. Individual differ in the amounts of cytokines secreted in response to the triggering stimulus due to the cytokine polymorphisms and some polymorphisms appear to have consistent alteration in cytokine production. Studies are being conducted to determine whether associations exist between cytokine gene polymorphisms and susceptibility to particular diseases. The positive associations with cytokine SNPs in human diseases have been described.

The expanding list of candidate genes linked with GVHD includes cytokines, chemokines and their receptors. The physiopathology of GVHD provided the rational to prioritize which gene to study first. The main cytokine gene polymorphisms that have been linked to GVHD or transplant-related mortality (TRM) include TNF, IL-10, IL-6, IFN-g, IL-1 and TGF-b, and studies are being conducted on IL-2, IL-4, IL-13 and CTLA4 amongst others.

Summary

The question arises as to what is the most efficient method for gathering this information in these new areas of immunogenetics. Immunogenetic profiling can be defined as the process of extrapolating the information encoded in one's genetic makeup, which will help unlock the mystery of the immune response. With increasing evidence concerning the complexity of immune polymorphisms and the significant role these polymorphisms play in the immune response, it is imperative to gather not only the expected information but also the hitherto unknown information.

As the diversity of the HLA system and other similar systems continues to emerge, we may expect further evolution in the immunogenetics field.

With the rapidly expanding wealth of genetic, biological and functional information, we are faced with the challenges of making scientific complexity more productive and optimizing its translation into medicine and public health. The expanding knowledge could benefit from an integrated systems biology approach. MHC/HLA systems biology will foster the emergence of a truly scientifically based holistic pathway for MHC/non-MHC immunogenetics.